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Effect of Ethanol on the Rat Gastrointestinal Cholinergic Enzymes

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Key Words. Acetylcholinesterase · Choline acetyltransferase · Ethanol · Gastrointestinal tract · Diurnal

Abstract. Male Sprague-Dawley rats weighing between 180 and 220 g and maintained under controlled lighting and temperature conditions were used in this experiment. Animals were given ethanol (3 g/kg, p.o.) 24 h after fasting. One group was given ethanol at 10.00 h (light phase) and the other at 22.00 h (dark phase). One hour later, the treated animals with their proper controls were sacrificed and the mucosa of the stomach, duodenum, ileum and colon were separated and assayed for choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities. Data obtained indicate that the administration of ethanol resulted in significant decline ($p < 0.01$) in ChAT activity in the stomach and the colon during the light phase. A significant increase ($p < 0.01$) in ChAT activity was also noted in the ileum during the dark phase. There was a significant decrease ($p < 0.01$) in AChE activity in the stomach during the dark phase. The administration of ethanol also resulted in a significant decline in AChE activity ($p < 0.05$) in the duodenum and the colon ($p < 0.01$). The results obtained indicate that the gastrointestinal changes caused by ethanol administration may be related to changes in the cholinergic enzymes of the mucosa of the gastrointestinal tract.

Introduction

Evidence has been presented by many investigators suggesting that ethanol inhibits the neuronal release of acetylcholine (ACh). *In vivo* studies showed that low doses of ethanol reduce the release of ACh into cerebral cortical cups and push-pull cannulas in the midbrain [8, 19]. It has been shown that *in vitro* administration of ethanol diminished synaptic transmission through the superior cervical ganglia [14]. In addition, ethanol was found to inhibit the spontaneous release of ACh from brain cortical slices [3]. In comparison to other transmitters, it has been shown that acetylcholine is the most sensitive neurotransmitter to inhibition by ethanol [3].

The inhibitory effect of ethanol on electrically stimulated release of ACh is most likely an effect related to the cholinergic neurons, since no other neuronal system has been described to have the ability to accumulate choline at low concentrations, to convert it into ACh and to store it in synaptic vesicles for release [3]. As further support for cholinergic involvement in the effect of ethanol in the brain, it was shown that an acute dose of ethanol does increase the steady-state concentration of acetylcholine in the rat brain [10, 12]. Moreover, ethanol was found to alter the high affinity of choline uptake in the rat brein [11, 12].

Changes in the brain cholinergic enzyme activity have also been reported [22]. It has been reporĵd that after 4 days of ethanol treatment, there was an increase in choline acetyltransferase (ChAT) activity in the striatum as compared to untreated controls [6]. On the other hand, a decrease in both ChAT and acetylcholinesterase (AChE) activities in the whole brain after 21–35 days of ethanol treatment has been reported [21]. Moreover, it was reported that ethanol and barbiturates behave similarly with regard to the cholinergic activity in the CNS, although the effects of acute and chronic treatments were different [18].

It has been demonstrated that intraluminal ethanol, in concentrations normally found in the duodenojejunal lumen after moderate drinking, causes mucosal morphologic lesions and alterations in the mucosal microcirculation [9, 17]. Ethanol was found to cause an alteration in jejunal morphology and fluid secretion, and that these changes are accompanied by an increase in mucosal arteriolar blood flow, shunting of blood through non-capillary microvessels, hypermia, hemoconcentration, and enhanced the loss of plasma protein into the gut lumen [2]. Ethanol at 20% concentration decreased acid secretion in the stomach to one third of control values [20].

The purpose of the present investigation was to study the effect of oral ethanol administration on the key enzymes of the cholinergic system of different gastrointestinal regions. We studied the diurnal effect of ethanol on ChAT (EC 2.3.1.6) and (EC 3.1.1.7). Knowledge of the effect of ethanol on these cholinergic enzymes will enable us to understand the mechanism of action of ethanol on the gut.

Materials and Methods

Male Sprague-Dawley rats weighing 180–220 g and purchased from Southern Animal Farms (Prattville, Ala.) were used in this study. They were adapted to a temperature of 21 ± 1 °C in an environmental chamber equipped to provide 23.25 cd/m² of cool, white fluorescent light for a minimum period of 2 weeks. The light period in the chamber was automatically timed to last from 08.00 to 20.00 h followed by a 12-hour dark period. Standard pellet diet (Purina, St. Louis, Mo.) and water were provided *ad libitum.*

In this experiment, 24 animals were randomly divided into four groups. In the first group, the animals were given 3 g/kg of ethanol (20% w/v) in saline orally at 10.00 h. The second group, the control group was given saline. The third group had the same treatment as the first, but ~~were~~ given ethanol at 22.00 h. The fourth group was given saline and served as a control for the third group.

One hour after each treatment all animals were sacrificed by decapitation. The stomach, duodenum, ileum and colon were separated on ice and the mucosa of each was gently scrapped from each region by means of a scalpel. The mucosa of each was used for the assay. AChE activity was assayed using a spectrophotometric method [7]. Each tissue was homogenized (1% w/v) in 0.1 *M* ice-cold buffer (pH 7.4) containing 0.1% Triton X-100 (Sigma). AChE activity in each homogenate was immediately determined and expressed as nano moles of substrate (acetylthiocholine iodide, Sigma) hydrolyzed per minute per milligram of tissue. For ChAT activity, each tissue was homogenized (1% w/v) in 0.5 *M* ice-cold phosphate buffer (pH 7.0). ChAT activity was determined using a spectrophotometric method [4] and was expressed as micromoles of acetyl CoA sulfhydril formed per minute per milligram of tissue.

Data were statistically analyzed using one-way analysis of variance [23]. The significance of the difference between the means of various tissues were determined using the LSD test [23].

Results

Figure 1 shows that oral ethanol administration resulted in a significant decline ($p < 0.01$) in AChE activity of the stomach in the dark phase with no significant change in the light phase.

Thgere was a significant decline ($p < 0.05$) in AChE activity in the light phase of the duodenum. Figure 2 shows that there was no significant change in the dark phase.

The effect of ethanol administration on the AChE activity of the ileum is presented in figure 3. There was no significant difference between control and ethanol-treated groups in both the light and dark phases.

In figure 4, it is shown that there was a significant decline ($p < 0.05$) in AChe activity of the colon in the light phase with no significant change in the dark phase.

A significant decline ($p < 0.01$) of ChAT activity was seen following ethanol admoinistration in the stomach during the light phase. There was no significant change of ChAT activity in the dark phase (fig. 5).

Figure 6 shows that there were no significant changes in ChAT activity of the duodenum following ethanol administration during both the light and dark phases.

In the ileum, as depicted in figure 7, there was no significant change in ChAT activity of the ileum during the light phase, whereas there was a significant increase ($p < 0.01$) in the dark phase following ethanol administration.

Figure 8 represents the effect of ethanol on the activity of ChAT in the colon. There was a significant decline in ChAT activity (p < 0.05) in the light phase with no significant effect during the dark phase.

Discussion

Results of this study indicate that the effect of ethanol on cholinergic activity varies in different parts of the gastrointestinal tract. This is seen by variations in the activity of the enzymes, AChE and ChAT, used as markers for cholinergic activity. These activities also vary diurnally. It has been shown that levels of plasma ethanol display a diurnal rhythm [24]. Thirty minutes after the plasma level occurred during the dark phase at 02.00 h and the lowest level occurred during the light phase at 10.00 h. In all tissues studied in the present experiment, there were differences in the effect of ethanol on cholinergic enzyme activity during the light and dark phases except in the effect on AChE in the ileum and ChAT in the duodenum. These effects could be attributed to the activity versus inactivity phases of the animal.

Results of this experiment are comparable to those of other investigators on ethanol-induced hypothermia [24]. These investigators found ethanol-induced hypothermia varied in a circadian manner with the greatest effect being observed at 10.00 h and the least effect at 06.00 h.

A positive correlation between lowered body temperature and the severity of gastric lesions has been suggested [1], and the marked fall in body temperature during stress affects the induction of parasympathetic stimulation resulting in ulceration [25]. The parasympathetic discharge also has been implicated in the ulcerogenic process [5]. During stress, like in alcohol consumption, experiments have shown an alteration of the central cholinergic activity in the spontaneously hypertensive rats. Since cholinergic activity is a major activator of gastric secretion, which in turn affects the gastric mucosa, the present work lends support to this idea. Moreover, elevation of gastric acid secretion without a parallel increase in mucosal blood flow will result in ulceration and changes in the local microcirculation of the gastric mucosa [13].

More significant changes were found in cholinergic activity during the light phase as compared to the dark phase. It is known that rats are nocturnal. Therefore, they are more active at night. Faster metabolism at night due to greater activity might account for less changes in cholinergic activity during the active period.

Circadican rhythm effects of ethanol has already been reported for epinephrine and norephinephrine. Alcohol intake resulted in a disorder of the sympathoadrenal system over 2 days after the circadian rhythm of catecholamine excretion changed [15]. In an effort to show the effect of phase-shift in the photoperiod of ethanol effect, activity counts showed an inconsistent profile followed by a reduced frequency of occurrences in mice housed in complete darkness compared to the controls [16].

Data from the present investigation indicate that ChAT and AChE activities in several GI tract regions are responsive to ethanol. Although the inhibition of ChAT activities noticed in some regions of the GI tract might be related to the inhibitory effect of ethanol on ACh release [2, 8 19], this study does not address the mechanism(s) by which ethanol can affect this cholinergic enzyme. Obviously, a study dealing with ACh level and turnover rate would further our understanding of the action of ethanol on the GI tract.

Acknowledgment

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Fig. 1. Effect of ethanol (3 g/kg) on AchE activity of the stomach during the light and dark phases. Each bar represents the mean + SEM of 6 animals. Asterisk indicates that the treated group was significantly different from the control group ($p < 0.01$).

Fig. 2. Effect of ethanol (3 g/kg) on AChE activity of the duodenum during the light and dark phases. Each bar represents the mean ± SEM of 6 animals. Asterisk indicated that the treated group was significantly different from the control group ($p < 0.05$).

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Fig. 3. Effect of ethanol (3 g/kg) of the AChE activity of the ileum during the light and dark phases. Each bar represents the mean ± SEM of 6 animals.

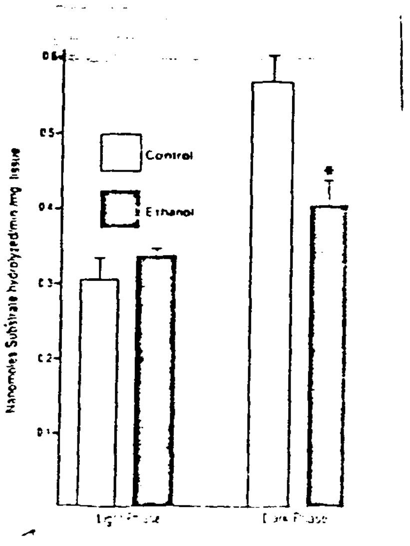
Fig. 4. Effect of ethanol (3 g/kg) on AChE activity of the colon during the light and dark phases. Each bar represents the mean ± SEM of 6 animals. Asterisk indicates that the treated grop was significantly different from the control group ($p < 0.01$).

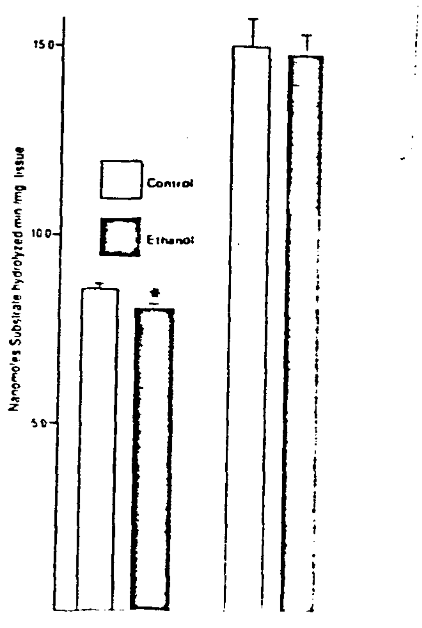
Fig. 5. Effects of ethanol (3 g/kg) on ChAT activity of the stomach during the light and dark phases. Each bar represents the mean SEM of 6 animals. Asterisk indicates that the treated group was significantly different from the control group ($p < 0.01$).

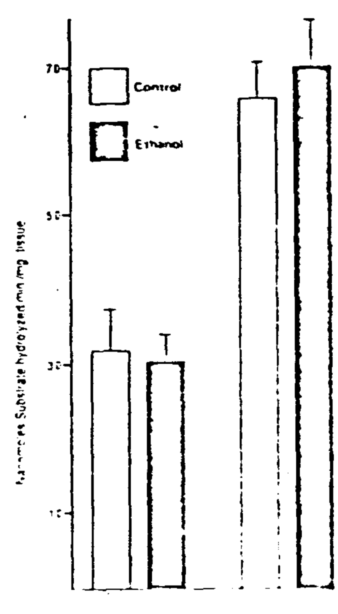
Fig. 6. Effect of ethanol (3 g/kg) on ChAT activity of the duodenum during the light and dark phases. Each bar represents the mean ± SEM of 6 animals.

Fig. 7. Effect of ethanol (3 g/kg) on ChAT activity of the ileum during the light and dark phases. Each bar represents the mean ± SEM of 6 animals. Asterisk indicates that the treated group was significantly different from the control group ($p < 0.01$).

~~Fw3~~Fig. 8. Effect of ethanol (3 g/kg) on ChAT activity of the colon during the light and dark phases. Each bar represents the mean ± SEM of 6 animals. Asterisk indicates that the treated group was significantly different from the control group ($p < 0.01$).







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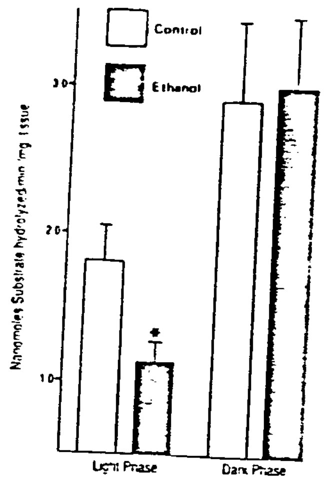

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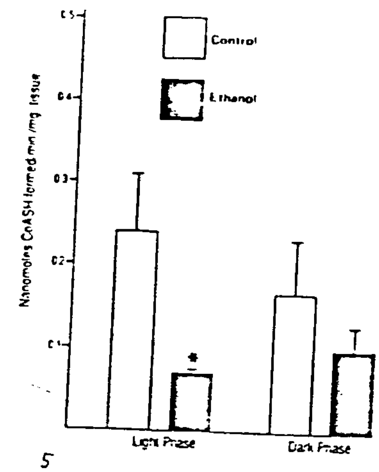
COLON



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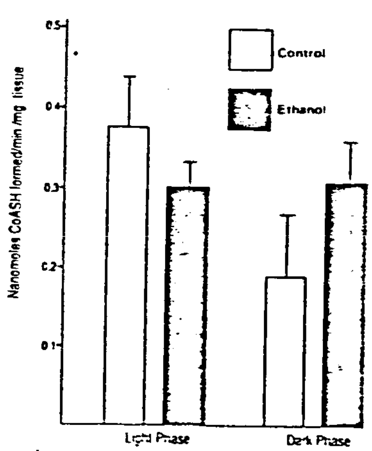
STOMACH



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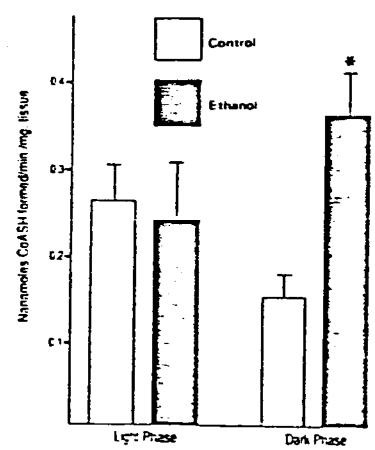
DUODENUM



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ILEUM